THE "PYLORIC GLAND" OF THE ASCIDIAN BOTRYLLUS—AN ORGAN OF EXCRETION?

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INTRODUCTION.

This contribution deals with the anatomy and physiology of an organ found in most of the groups of the Tunicata. This organ is usually composed of a system of fine tubes which ramifying over the walls of the intestine, are joined to some portion of the stomach by one or more ducts. Although of general occurrence in the Ascidiacea and Thaliacea, yet there has been no concurrence of opinion as to its function. In consequence of this fact hardly two authors have referred to it by the same name. Hence we have this organ figured as (1) glandes diverses (Savigny, 1810)[1] and referred to, if we leave out literal translations as, (2) liver (Hancock, 1866) (Krohn, 1852) (Milne-Edwards, 1841),[2] (3) glande annexe du tube digestif (Chandelon, 1875) (v. Winiwarter, 1896), (4) glandola epato-pancreatico (Della Valle, 1881), (5) lacteal system (Lister, 1834) (Huxley, 1851) (Ritter, 1896), (6) intestinal gland (Herdman, 1882) (Maurice, 1888), (7) lacunes stomaco-intestinales (Roule, 1884), (8) glande stomacale (van Beneden et Julin, 1884), (9) darmumspinnende Drüse (Seeliger, 1882) (Dahlgrün, 1901) (Isert, 1903), (10) organe réfringent (Giard, 1872) (Pizon, 1893) and (11) glande pylorique (Lacaze-Duthiers et Delage, 1889) (Willey, 1893).

With such a choice of names what shall we call the organ in question? Since the name pyloric gland is short and sufficiently non-committal and has been dignified by usage, we will use it in preference to the others.

The following study is an outgrowth of one that the writer has been at work on for the past two years, and as it has developed into slightly other lines than was originally planned, he has taken this excuse to make a separate paper of this portion. The work

[1]See Chandelon, 1872.

[2]See Giard, 1872*a*.

as a whole was begun in the zoölogical laboratory of the University of Pennsylvania. It was continued at the Zoölogical Station at Naples, at the Fisheries Laboratories at Woods Hole, and Beaufort, and the following part of it completed in the Zoölogical Laboratory of the University of Pennsylvania. At this point the writer wishes to express his great thanks to the Carnegie Institution for the use of one of their tables at the Naples Laboratory, to the authorities of the station for their many kindnesses and hospitality, to the United States Commissioner of Fisheries for the use of a table for two weeks at Woods Hole, and one for two weeks at Beaufort, and also to the directors of those stations, Dr. F. B. Sumner and Mr. H. D. Aller in particular.

Although for this study most of the living material was procured in the salt-water tanks of the vivarium of the University where *Botryllus* colonies have been established for many years, yet the wealth of material preserved in Naples and Woods Hole has often been called into requisition while living material of other families of ascidians were studied at Beaufort.

According to Bancroft ('03) there is but a single species of *Botryllus* found in the north Atlantic Ocean and its extensions. Many have been described, but they are found to be based on color variations and habit of growth depending partly on the age and partly on the physiological state of the colony. The writer having worked at both Naples and at Woods Hole supports the view of Bancroft and considers that *Botryllus schlosseri* (Pallas) Savigny, is the form represented on both sides of the ocean.

The material was fixed in Flemming's solution, in corrosive sublimate, sublimate acetic, formol, etc. The best results were procured with Flemming's solution. Sections were cut 6 μ and stained in Delafield's hæmatoxylene and eosin. However, most of this study was made on the living animals and sections were used only to check up the results.

Morphology.

The alimentary tract of *Botryllus* is of the typical ascidian type and may be represented by the letter U of which one arm will be the œsophagus and stomach while the other is represented

by the intestine and rectum (Fig. 1). In the angle between the two arms lies a small blind sac, an out-pocket from the pyloric end of the stomach. Its walls are thick and glandular, similar to the walls of the stomach, but the cells that compose it do not contain the secretion that gives the stomach its characteristic yellow color.

This organ was called by Lahille ('90) the pyloric cœcum. It is into this sac, at the point where it enters the stomach, that the duct of the pyloric gland empties.

If we follow the duct from the point where it enters the pyloric cœcum and trace it to the intestine we will find it divide just before reaching that organ, sending a branch both to the right side and to the left side. At once on reaching the walls of the intestine both branch many times, finally ending in blind bulbs or ampullæ. However, all the branches do not end thus, but a few—not more than five or six—proceed half way to the anus, often without branching again. These tubes do not end in ampullæ. As they reach the region of the rectum in some cases, we will for convenience refer to them as rectal tubules.

It is very easy to study the organ in the living *Botryllus* and indeed it is possible in that way to see much more than can be observed in the preserved material, either in sections or in surface view. When a cormus is removed from its substratum and placed on a microscopic slide, the stomach and intestine can be teased out with a pair of needles under a dissecting microscope. The cormus is then removed from the slide, a drop of sea water added and the whole covered with a cover glass. The writer found artificial light in the shape of a Welsbach burner, a Zeiss apochromatic 2 mm. objective, and compensating oculars 8 × and 12 ×, necessities in the present study.

In the living tissue the tubes and bulbs appear highly refractive. In some cases it is quite easy to see the nuclei and even the chromatin in the nuclei. In many cases cell boundaries are quite clear and the presence of cell granules is easy to determine.

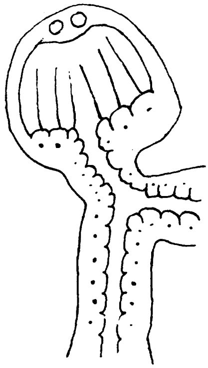
The ampullæ are bounded by a rather flat epithelium, the cell walls of which contain often refractive granules of a yellow color. The cells bear long whip-like flagella, but it is difficult to determine if all are so provided. These flagella soon lose their move-

ment after being placed on a slide, although the writer has observed them beating for five hours after the alimentary tract was removed from the organism. Fig. 2 represents the character of the epithelium and flagella in two adjoining ampullæ.

In *Botryllus* this organ was reported ciliated by Della Valle ('81) who wrote, p. 458: "La struttura intima di questa glandola è simplicissima trattadosi d'un semplice epitelio, che io ho veduto sempre sfornito di cigli vibratili." He gives no figure.

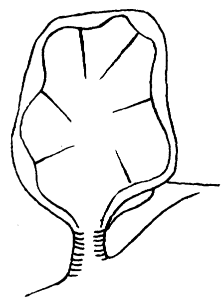
Pizon ('93) particularly mentions that he is unable to verify Della Valle and finds the lumen of the ampullæ unciliated. There are three other cases in which the pyloric gland has been found ciliated—Chandelon ('75) in *Perophora*, text Fig. 1, Uljanin ('84) in *Doliolum*. text Fig. 2 and Isert ('03) in *Microcosmus*. The

FIG. 1.



After Chandelon.

FIG. 2.



After Uljanin.

Ampullæ of the pyloric gland as seen in *Doliolum* and *Perophora* respectively.

latter found the ducts and believed the ampullæ were ciliated too, although he could not see it. The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much if not more could be seen in the living.

Although the protoplasm of the walls of the ducts and ampullæ seems to be clear and refractive in the living animal, yet there are here and there yellowish granules in the cells. These are exceedingly minute, .1 to .2 μ in diameter, and are found in that portion of

the cell nearest to the lumen of the gland. One rarely sees more than a single granule in a cell. Among these small granules are larger ones, .5 to .8 μ, distinguished from them by being more refractive. In character they seem much like the brown concretions found in certain blood cells (Fig. 18). However, these concretions cannot be found in the preserved material, so the supposition is that they are composed of a material that is not coagulated by the killing fluid, this material being of the nature of a secretion. The concretions in the blood cells are not easily dissolved by any ordinary reagents used in microscopic technique therefore they are found in the preserved material.

At Beaufort the writer had the good fortune to be able to examine the ampullæ of the living *Amaroecium stellatum*, *Perophora veridis*, *Ascidia* sp., *Styela plicata* and *Molgula manhattensis*. All had flagella in the pyloric gland. In *Amaroecium* the flagella were similar to those found in *Botryllus*. In *Perophora* some ampullæ were like those found in *Botryllus* (Fig. 22), but others had exceedingly few cilia which were directed toward the mouth of the tube and not away as in Chandelon's figure (Fig. 21). Since the writer was able to observe movement in all the cilia in this ampulla their direction can be determined to a certainty. The walls of the ampullæ of *Ascidia* showed no essential character different from that of *Botryllus* (Fig. 19). In the cells of the gland of *Styela* (Fig. 24) is found the yellow secretion just as Isert described it in *Microcosmus* and in the lumen of the gland are found globules of it. In *Molgula* the organ is typical (Fig. 23). Since so many diverse families of ascidians show flagella in the lumen of the pyloric gland, the writer believes that if carefully looked for this organ in all tunicates will be found to bear flagella.

The walls of the tubules are similar to those of the ampullæ except that the cells are more cuboid (Fig. 3), and the flagella shorter. No granules are to be detected in the lumen of the tubes of *Botryllus* such as Isert found in the ducts of the pyloric gland of *Microcosmus*, and as the writer has found in the ducts of the gland in *Styela*.

When we compare the rectal tubules with the other portions of the organ in *Botryllus* several things may be noticed. Among

these things our attention is particularly called to the relative fewness of flagella and nuclei in the terminal portions of the tubules. Figs. 4–13 show flagella while Figs. 14 and 15 show nuclei. Although it is not possible to distinguish cell boundaries either in the preserved material or in the living tissue, yet the fewness of the nuclei might suggest the possibility that the cells composing this part of the gland have intracellular lumens. Again the terminal ends of these tubes exhibit two different types. We may have those unsegmented with a very small lumen, 2–6 μ (Figs. 8–12, 14 and 15), or we may have segmented tubes with a much larger lumen, 4–10 μ. In the first type of tube our attention is at once attracted to the terminal end of the tube. In most cases there seems to be a very thin place in the walls of the tubes. This thin place may be formed in three ways: (1) by the lumen of the tube approaching the exterior (Figs. 8, 14, 15), (2) by a cup-like depression in the end of the tube (Figs. 9-12, 15), (3) by a vacuole in the wall of the tube which does not communicate either with the exterior or the interior. To these three cases there is a fourth effect that the writer has observed. He thought that he could see tubes less than a micron in diameter that formed a direct communication between the interior of the tube and the blood space. The structures found at the end of the rectal tubules are so small that what we may interpret as a duct may be nothing more than a division between two cells—or a nucleus—both of which look clearer in preserved material and in the living tissue than the cytoplasm of the cell. The cup-like depression at the end of the tube suggests the organ of Boveri as found in *Amphioxus* or perhaps a nephridial funnel. The writer has searched the neighborhood about the ends of the tubes to see if he could find solenocytes as described by Goodrich ('09) in *Amphioxus* but without result. Again he has watched particles in the blood in the neighborhood of the possible opening, yet in no case has he been able to observe such a particle enter the tube. In this connection the experiment of Kupffer ('72) is interesting. He says (p. 381): "Mir ist es auch bei *Ascidia canina* gelungen. dieses System wenigstens partiell vom Herzen aus zu injiciren. Die Injectionsmasse war in mehrere der blinden Anhänge eingedrungen. Solche blinde kolbige Anhänge sind auch nichts Neues

im Gefässsystem der Ascidien. Man findet dasselbe an den colonialen Gefässen in der gemeinsamen Tunica der Synascidien. Ich halte daher das Ganze für einen besonders enwickelten Theil des Circulationsapparates, dem wohl neben der Resorption des Chymus noch andere Functionen zukommen."

If *Ascidia canina* should have open communication between the lumen of these tubes and the blood cavity it would easily explain how Kupffer found the injection mass in the lumen of the gland. Since no communication has been demonstrated, it would be easier to explain the result of Kupffer in the light of the writer's own experiments with indigo carmin on *Styela* (p. 43). With the present evidence before us we cannot assume that there is any communication between the blood spaces of *Botryllus* and the lumen of the pyloric gland.

The cases in which cilia have been seen in the ampullæ were all observed before the modern methods of microscopic technique were evolved. Since then we have been carried away by the use of dead material when in many cases, perhaps, as much, if not more, could be seen in the living.

Pizon ('93) has studied the origin of this organ both in the tadpole and bud of *Botryllus*. Of this study the writer has verified the results and can but accept the conclusion of Pizon ('93) that in both cases the origin of the pyloric gland is from the endoderm by a simple diverticulum of the gut. This agrees perfectly with Van Beneden and Julin ('84 and '86) in *Phallusia*, Lefevre ('98) in *Perophora*, Ritter ('96) in *Goodsiria*, Seeliger ('82) in *Clavelina*, Uljanin ('84) in *Doliolum*, etc. In all cases it arises as an out-pocket of the stomach.

Experiments.

The smallness of the pyloric gland in *Botryllus* and the fineness of the tubes and ampullæ as found in the larger ascidians, together with the close application of the gland to the walls of the intestine, to the reproductive organs or to the renal vesicles, would forbid, in any form that has yet been available to the writer, direct physiological determinations. It is due to this that the nature of the organ has been problematical. To be sure Henri ('03) claims to have isolated the gland in *Salpa*, but as yet the

writer has been unable to procure the form in question. As no direct experiments on the nature of the fluid contained in the ducts and ampullæ have in this case been possible, the writer has resorted to indirect means—that of the use of certain in-vitam stains.

In the use of these stains the writer has not proceeded far and hopes at another time to undertake a fuller discussion of their significance. Suffice it to say that certain dyes, when introduced into the blood of a living organism in solution, have affinities for formed substances in the cells of certain tissues, as methylene blue in nervous tissue. Others, such as neutral red, act as indicators, telling us whether a given substance has an acid reaction or not, while still other dyes are segregated out of the blood as solids and deposited in cavities often connected with the exterior.

Following the experiments of Chrzonszezewsky ('64), Heidenhain ('74)[1] by injecting certain dyes, principally indigo carmin and ammonium carminate, into the veins of vertebrates came to the conclusion that the former dye was excreted by the Malpighian tubules of the kidney, while the glomeruli excreted the carminate. Kowalewsky ('89) carried this idea into his experiments on invertebrates, concluding that renal cells show either acid or alkaline reaction which determines the character of the secretion. However, Schmidt ('91)[2] has shown this idea false, as both ammonium carminate and indigo carmin may be excreted by the same organ. Nevertheless, it is a rather general characteristic of renal organs that they excrete carmin in some form.

In this study of *Botryllus* the writer has placed colonies in neutral red, in Bismarck brown, in ammonium carminate and in indigo carmin, studying the reaction of the pyloric gland to these dyes. Neutral red in concentrations rendering the sea water a pale yellow, stains the secretion in the cells of the organ an intense red and colors the liquid in the lumen of the ducts and ampullæ also. The probable significance of this is that the secretion has an acid reaction. Bismarck brown coloring the water much like that of the neutral red, stains the granules brown and

[1] Cited from Bruntz, '03.

[2] Cited from Bruntz, '03.

in contrast to the blood and to the sea water, the contents of the lumes of the tubes and bulbs is very brown. There are two alternatives by which to interpret these two experiments—either these stains act as indicators or they are actually concentrated in the lumen of the organ being separated from the blood by the cells of the walls of the tubes and ampullæ. Beyond certain leucocytes located in the neighborhood of the organ which seem to collect the dye, the only other cells that take up the ammonium carminate and indigo carmin are the vacuoles in the intestinal cells. A cross-section of the intestine has the shape of a rectangle (Fig. 17), the ends of which are made up of an epithelium of flat cells bearing cilia, while the sides are thick and are composed of two sorts of columnar cells, ciliated and glandular (Fig. 14). The gland cells under ordinary condition contain a clear liquid. It is the vacuoles of these gland cells which stain slightly in ammonium carminate and indigo carmin. The significance of this will be discussed later on.

Both *Molgula* and *Ascidia* were treated with indigo carmin at Beaufort but the pyloric gland showed no reaction to them.

In *Styela*, however, indigo carmin in concentrations as shown in the table gave in every case a characteristic reaction. The material in question were rather small *Styela* 20–30 mm. The large ones required too large receptacles.

Experiment	1.	100 c.c. saturated sol. of indigo carmin in sea water.		100 c.c. sea water		4 days.
"	2	100 c.c.	do.	100 c.c.	do	2 '
"	3	100 c.c.	do	100 c.c.	do	4 "
"	4	200 c.c.	do			2 "

The animals in experiments 1, 2 and 3 lived, in experiment 4 they died.

When the animals were examined large blue concretions were found in the ampullæ and ducts of the pyloric gland (Figs. 25 and 26). These concretions gave that portion of the intestine covered by the pyloric gland a blue color. The writer considers that the indigo carmin was excreted from the blood into the canals of the gland.

Discussion.

Turning now to the character of the pyloric gland in other tunicates, we may as well begin with the Larvacæ. Although this organ is absent in most of the genera, it seems to be represented at least in a rudimentary form in two described by Chun ('81) from deep water of the Mediterranean Sea—*Stegasoma* and *Megalocercus*. Here we have a diverticulum of the gut which may be a possible homologue of the pyloric gland or at least to the pyloric cœcum. However, in no case is the organ developed as it is in the other orders of tunicates.

Our present knowledge of the pyloric gland in the Thaliacea and Ascidiacea can best be presented in tabular form. The table in question does not pretend to be complete but gives in condensed form the observations of various investigators.

Family.	Genus.	Authority.	No. of Ducts.	Type.	
Doliolidæ	*Doliolum*	Uljanin ('84)	1	Dendritic	Ciliated.
Salpidæ	*Salpa*	Chandelon ('75)	2	Reticular	——
Pyrosomidæ	*Pyrosoma*	Huxley ('59)	1	"Ramifying"	——
		Seeliger ('89)	1	Dendritic	——
Polyclinidæ	*Fragaroides*	Maurice ('88)	1	Reticular	Unciliated.
	Amaroecium	Author	—	——	Ciliated.
Distomidæ	*Distaplia*	Della Valle ('82)	1	Reticular	——
Botryllidæ	*Botryllus*	Della Valle ('82)	1	Dendritic	Ciliated.
		Pizon ('93)	1	Dendritic	Unciliated.
		Author	1	Dendritic	Ciliated.
Polystyelidæ	*Goodsiria*	Ritter ('96)	1	Branching	——
Clavelinidæ	*Clavelina*	Seeliger ('82)	1	Dendritic	——
		Author	1	Dendritic	Ciliated.
Perophoridæ	*Perophora*	Chandelon ('75)	1	Dendritic	Ciliated.
		Author	1	Dendritic	Ciliated.
Phallusidæ	*Phallusia scabra*	v. Winiwarter ('96)	2	Reticular	——
	Ascidia sp.	Author	—	——	Ciliated.
	Corella	v. Winiwarter ('96)	5–11	Reticular	——
Cynthiadæ	*Microcosmus*	Isert ('03)	1	Reticular	Ciliated.
	Polycarpa	Lacaze-Duthiers ('89)	1	Dendritic	Unciliated.
	Styela rustica	Wagner ('85)	1	Reticular	——
	Styela plicata	Author	1	——	Ciliated
	Styelina	Lacaze-Duthiers ('89)	1	Dendritic	——
Mulgulidæ	*Molgula* sp.	Author	—	——	Ciliated.

As far as this organ has been particularly described it is much the same in all families. The usual number of the ducts is one, but there may be more. The tubules are either dendritic or

form a network over the intestine. All the tubes, ducts and ampullæ that have been examined carefully have been found to be lined with a ciliated epithelium. Moreover, it is worthy of note, perhaps, that Huxley, ('51) and Della Valle ('81) have observed a bladder-like swelling of the duct in *Didemnum*. Todaro figures the same for *Salpa*. A type of gland differing slightly from the ones referred to above was described by Julin ('04) in *Archiascidia*. The single duct was short, and branched into but six tubules which did not branch on the intestine but ran parallel to one another almost all the way to the anus. There were no typical ampullæ. These tubes can best be compared to the rectal tubules described above for *Botryllus*.

As the writer mentioned in the introductory paragraph great difference of opinion exists as to the function of this organ. To be sure, few authors have ventured to strongly support one idea and most have been quite reserved in their conclusions, yet it is of sufficient interest to warrant the writer's reviewing their opinions briefly. Not considering those views that were based clearly on misconceptions cf. Vogt ('54), as to the structure of the organ, we will take up a few of the others. Really when Hancock ('66) called the organ in question a true liver much can be said to support the view. Its relation to the blood supply plainly recalls that of the vertebrate liver. Indeed with what we know at present of the organ, it would be very difficult to refute this idea, particularly as the vertebrate liver has been known to excrete carmin.

Chandelon ('75), Della Valla ('85) and von Winiwarter ('96) and Isert ('03) consider, after thoroughly reviewing the subject, that the function is digestive. Henri ('03) has other than morphological evidence. He says, p. 765: "En faisant des macérations de cette glande pylorique, on obtient un liquide riche en amylase, il ne digére ni l'albumine, ni la fibrine; cette macération agit au contraire faiblement sur la gélatine. Cette glande contient donc bien des ferments digestifs. Les macérations des autres parties du corps de la *Salpe* donnent des résultats négatifs."

Kupffer ('52) and Roule ('84) by means of injections arrived at the conclusion that these tubes were part of the blood vascular system. Lister ('34), Huxley ('51) and after them Pizon ('93)

and Lefevre ('98) have agreed that it probably serves an absorbing function, something like the gastrovascular canals of Cœlenterata. Huxley ('51) asked: "Does this tubular system represent a hepatic organ or is it not more probably a sort of rudimentary lacteal system—a means of straining off the nutritive juices from the stomach into the blood by which these tubes are bathed?" It is very probable that the organ has a digestive function; there seems strong evidence to support that idea. But the direction of the cilia in the duct would forbid the conclusion of Huxley, etc., that the function is that of absorption.

There is yet another function that has been attributed to the pyloric gland. Kowalewsky ('74) was inclined from what he knew of the structure of the organ in *Peropora* to attribute to it urinary functions.

Krunkenberg ('80) says: "Ich finde sie als constantes, durch die Murexideprobe leicht und schön nachzuweisendes Product der als Nieren angesprochnen drüsigen Darmanhänge bei *Phallusia mentula*." This statement is based on a misconception. He did not distinguish that the pyloric gland and the renal vesicles were not part of one system. What he analyzed were the concretions which others have found to contain uric acid. This interpretation is supported by the fact that he could not find uric acid in the gland of *Ciona* and of *Cynthia*, neither of which have renal vesicles covering the intestine.

In *Salpa*, Todaro ('01–'02) described three pairs of diverticula from the alimentary canal that had the power of taking up carmin. The first pair was in the pharynx, the second pair in the œsophagus and the third pair the pyloric gland.

Let us now turn and inquire as to what organs have been previously described as possessing the power of elimination of waste products of metabolism from the body of tunicates. Roule ('84) makes the distinction between a kidney of excretion and one of accumulation. Through the investigations of Van Beneden ('46), Kupffer ('72), Lacaze-Duthiers ('74), Kowalesvky ('89) and Dahlgrün ('00), we have a knowledge of this latter type of organ at least in a few groups. The kidney accumulation may be said to consist of two types. In *Salpa*, *Ciona* and *Botryllus* it consists of blood cells containing brownish concretions. The second type

is composed of closed vesicles lined by an nonciliated epithelium which encloses a fluid in which are suspended one or more relatively large concretions. There may be many small vesicles as in *Ascidia* and *Ascidiella.* In *Cynthia* we have a few larger vesicles and in *Molgula* a single large one.

In a kidney of accumulation, the waste matter of the organism is stored up in the form of a solid which is freed from the organism only by death. Harmer ('92) has described such an organ in the ectoprocta and the kidney of accumulation can be found in several groups of animals. In the Tunicata other organs have been described as kidneys of excretion. Julin ('91) suggested that the neural gland had perhaps an excretory function. Metcalf ('00), on reviewing the subject of the neural gland, considers that there is no evidence to support the view of Julin. Roule ('84–'85) on the other hand, described about the opening of the deferent canal in *Ciona* a mass of pigment cells which according to this author is a kidney of excretion.

To the view that the pyloric gland is a kidney, there is one serious objection. This is the fact that Henri ('03) found in the pyloric gland of *Salpa* a diastatic ferment in great abundance. This would seem to be a strong argument in favor of a digestive function for the organ were it not that such a ferment is found in the kidneys of certain mammals, sparingly in the dog it is true but richly in the rabbit (Oppenheimer, '09).

We have in certain forms of tunicates a kidney of accumulation, but in nearly all groups a pyloric gland. The Appendicularia which are without it are so exceedingly minute that all their tissues, if not actually bathed by the sea water, are in close proximity to it, so that the need of special organs of excretion is not quite so urgent. Be that as it may the question naturally arises, has this tunicate organ any characters in common with the excretory organs of other groups of animals?

The shape and character of the terminal bulbs, and the ducts are parallelled in part by the multicellular ciliary flames found in the Nemertinea. A section of the terminal end of a duct in *Lineus* by Punnett ('11) shows a condition very similar to that of the ascidian. Each cell of the organ bears a single flagellum which is directed away from the blind end of the tube. That the

duct of the pyloric gland opens into the stomach and is derived in development from the entoderm is a condition characterizing no other excretory system out side of the Arthopods. However, since the vertebrate liver has been shown by Chrzonszezewsky ('66) to excrete carmin, and has morphologically the same position as has the pyloric gland of the ascidian and has a similar development, there is good reason to believe that the two are homologous. Willey has homologized the organ with the hepatic cœcum of *Amphioxus* which Hammar ('93) about the same time compared to the liver of the Craniota. Can we not conceive that in the hypothetical ancestor of the vertebrate the liver arose as an organ of excretion and in the tunicate it has retained more of those characters ?

Summary.

1. There are in *Botryllus* two sorts of terminations to the tubes that compose the pyloric gland, bladder-like ampullæ and long straight blind tubes—the latter we have called rectal tubules because in many cases they extend to the region of the rectum.

2. The ducts and ampullæ of *Botryllus* as well as *Ascidia*, *Styela*, *Molgula*, *Perophora*, *Clavelina* and *Amaroecium* are lined by cells bearing long whip-like flagella, the ends of which are directed toward the mouth of the duct.

3. Many of the rectal tubules have a termination difficult to interpret. This has the appearance, in most cases, of a cup-like depression in the end of the tube which seems to form a communication between the blood cavity and the lumen of the tube. In no case, however, could such a communication be demonstrated.

4. The direction in which the free ends of the flagella point indicates that the contents of the lumen pass toward the stomach and therefore the function of the organ is secretory rather than that of absorption.

5. Part of this secretion is probably found in the minute yellow globules found in the cells of the ducts and ampullæ. If these yellow globules represent a secretion, this is soluble in water and does not form masses in the lumen of the tube as in *Microcosmus* and *Styela*.

6. Bismarck brown and neutral red are concentrated in the

lumen of the organ in the form of a liquid while the indigo carmin is found concentrated in solid form in the gland of *Styela*.

7. In the tunicates in general no special kidney of excretion has been recognized. Although the gland in question may have other functions also, yet its structure and properties seem to indicate that it is the kidney of excretion of the tunicates, and is in turn homologous to the vertebrate liver.

LITERATURE.

Bancroft

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Description of Plates.

The figures were all drawn with a Zeiss microscope and apocromatic lenses. In making all the drawings a camera lucida and artificial light was used, in the case of *Botryllus* a Welsbach burner, and in the case of the other forms an ordinary oil lamp. With the exception of Fig. 1, Fig. 16 and Fig. 17 all were drawn at a magnification of 1,500 diameters, and have been reduced in reproduction to 1,000.

A = ampullæ.
bc = blood cell.
C = concretions of indigo carmin.
I = intestine.
Nu = nucleus.
O = Œsophagus.
PC = pyloric cœcum.
PS = peribranchial sac.
R rectum.
RT = rectal tubules.
RO = pyloric gland.
S = stomach.
V = vacuole in intestinal cell.

Explanation of Plate I.

Fig. 1. The stomach and intestine of *Botryllus*. × 52.

Fig. 2. Two ampullæ of the pyloric gland in *Botryllus*. This drawing shows the long whip-like flagella and also the granules in the cells. × 1,000.

Fig. 3. Portion of one of the main ducts. × 1,000.

Figs. 4–7. Optical section of a rectal tubule showing segmentation, granules, and flagella. × 1,000.

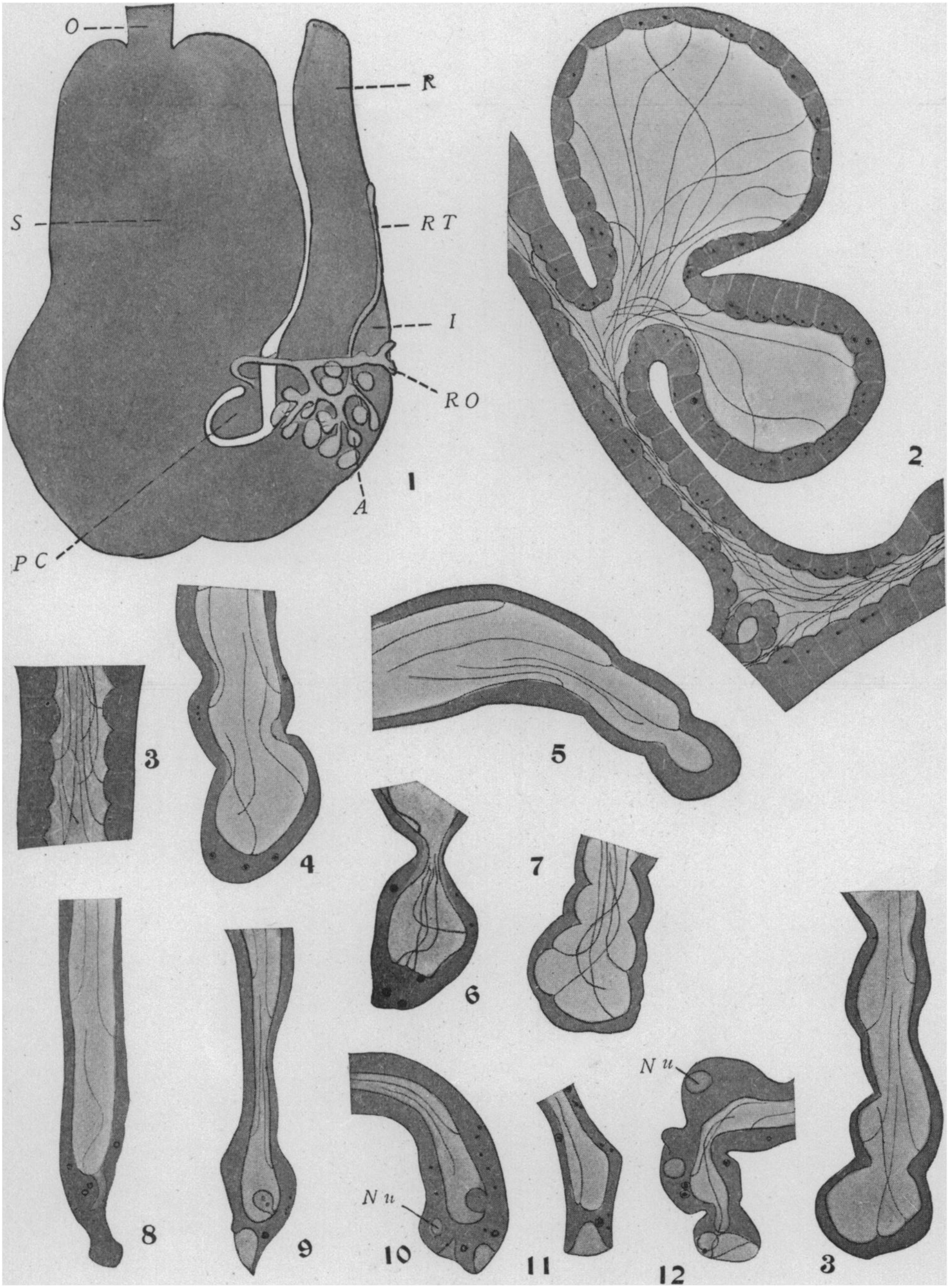
Fig. 8. Optical section of a termination of a rectal tubule showing a thin place in the wall of the tube. × 1,000.

Fig. 9. Optical section of a cup-like depression in the end of a tubule. × 1,000.

Fig. 10. Optical section of a tube with two cup-like depressions at the end. × 1,000

Figs. 11–12. Termination with a single cup-like depression. × 1,000.

Fig. 13. Optical section of a segmented tube in the walls of which there are no granules. × 1,000.



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EXPLANATION OF PLATE II.

FIG. 14. Section 6 μ thick of the termination of a rectal tubule of *Botryllus* which was fixed in Flemming's solution. This shows a thin place in the wall of the tube and also a vacuole. In this figure is also shown the relation of the tube to the walls of the intestine. The vacuoles in the intestinal cells are those which stained red and blue in ammonium carminate and in indigo carmin respectively. $\times$ 1,000.

FIG. 15. Drawing made from two adjoining sections of a rectal tubule, which show terminations with both a thin place and a depression. $\times$ 1,000.

FIG. 16. A section of a bud of *Botryllus* showing the origin of the pyloric gland as an out pocket to the stomach. $\times$ 320.

FIG. 17. Sections of the rectum of *Botryllus* fixed in absolute alcohol, glacial acetic acid and chloroform. This shows the relation of the tubes to the canal. $\times$ 150.

FIG. 18. Two living blood cells, in one of which the nucleus is visible in the other it is not. $\times$ 1,000.

FIG. 19. Optical section of ampulla of *Ascidia*. $\times$ 1,000.

FIG. 20. Optical section of rectal tubule of *Ascidia*. $\times$ 1,000.

FIG. 21. Optical section of ampulla of *Perophora*. $\times$ 1,000.

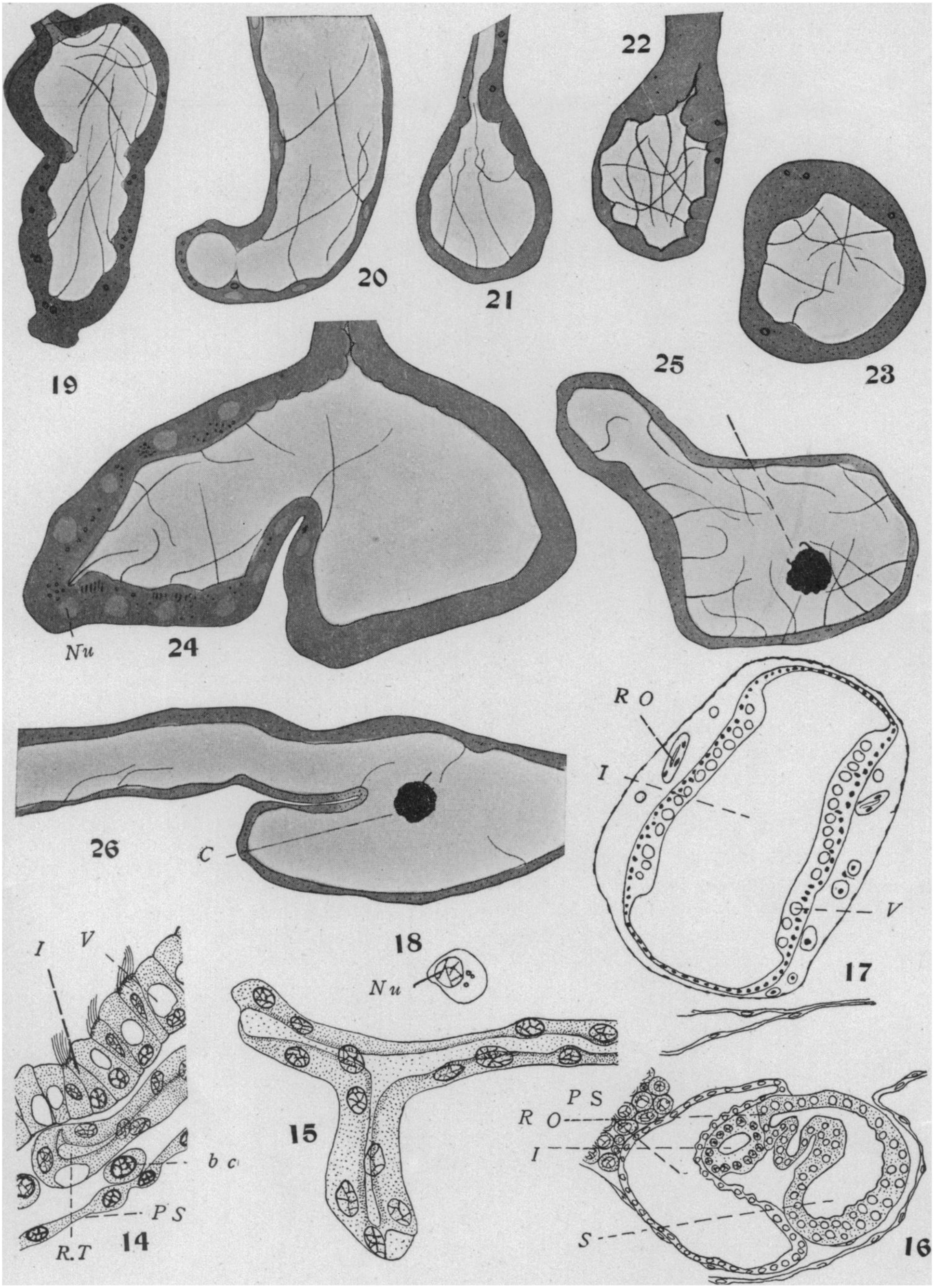
FIG. 22. Optical section of ampulla of *Perophora*. $\times$ 1,000.

FIG. 23. Optical section of ampulla of *Molgula*. $\times$ 1,000.

FIG. 24. Optical section of ampulla of *Styela*. $\times$ 1,000.

FIG. 25. Optical section of ampulla of *Styela*, containing a concretion of indigo carmin. $\times$ 1,000.

FIG. 26. Optical section of a tube of the refringent organ of *Styela* containing a concretion of indigo carmin. $\times$ 1,000.



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